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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignment and secondary structure of *Mycobacterium tuberculosis* adenylate kinase

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Biological context

Adenylate kinases (AKs) are ubiquitous small enzymes which catalyze the reversible transfer of the terminal phosphate group from ATP:Mg^{2+} to AMP and play a key role in the energetic metabolism and nucleic acid synthesis (Noda, 1973). Comparison of available AK sequences has suggested a first classification in two main forms: a short form, having about 190 residues, and a long one composed of 214–238 residues. Generally, the eukaryotic cytosolic enzymes are short variants, whereas the bacterial, yeast and mitochondrial AKs contain an additional segment, exposed to the solvent and highly flexible. However, several bacterial AKs were shown to have a short sequence while displaying a weak similarity with the cytosolic AK counterpart. This group, including AK from *Mycobacterium tuberculosis*, was proposed as a new subfamily of short bacterial variants (Munier-Lehmann et al., 1999). Several 3D structures of short (cytosolic) and long variants of adenylate kinases have been solved at high resolution by X-ray crystallography. In contrast, no detailed structural information is available for short bacterial variants.

We therefore focused our attention on *M. tuberculosis* adenylate kinase (AKmt) with the aim of obtaining its 3D structure in solution and of analysing the structure/function relationship. Tuberculosis continues to represent a major public health problem, due to the emergence of multiple-drug-resistant strains and to coinfection with HIV. As AKmt plays a critical role in *M. tuberculosis* survival (Kohiyama et al., 1966), and displays catalytic properties significantly different from the eukaryotic AKs (Munier-Lehmann et al., 1999), it may be expected that the bacterial enzyme might be a new target for antibacterial drugs.

In this Letter we report almost complete backbone and partial (over 80%) side-chain resonance assignment, as well as the secondary structure predicted by the NOE interactions and chemical shift analysis.

Methods and experiments

Uniformly labeled recombinant *M. tuberculosis* AK was overproduced in *Escherichia coli* strain Bli5/pHL20 (Munier-Lehmann et al., 1999) using M9 minimal medium containing 1.5 g/l 99% (^{15}N-ammonium sulfate and 3.0 g/l 99% (^{13}C)-glucose as the sole nitrogen and carbon source, respectively. The protein was purified as described earlier (Bârzu and Michelson, 1983).

NMR samples at a concentration of 1.2 mM (pH 7.1) were obtained by dissolving the lyophilized protein in potassium phosphate buffer (50 mM) in 95% $^{1}H_2O$/5% $^{2}H_2O$ or in 100% $^{2}H_2O$. 2D homonuclear, and double- and triple-resonance (HSQC, NOESY-HSQC, TOCSY-HSQC, HNCA and HN(CO)CA) NMR experiments (Wüthrich, 1986; Cavanagh et al., 1996) were performed on a Varian Unity-500 NMR spectrometer. The HCCH-TOCSY and triple-resonance 3D CBCA(CO)NH, HNCACB were acquired on a Varian Inova 750 MHz spectrometer (European SON NMR Large-Scale Facility, Utrecht). Proton chemical shifts (in ppm) were referenced relative to internal DSS and ^{15}N and ^{13}C references were set indirectly relative to DSS using frequency ratios (Wishart et al., 1995).

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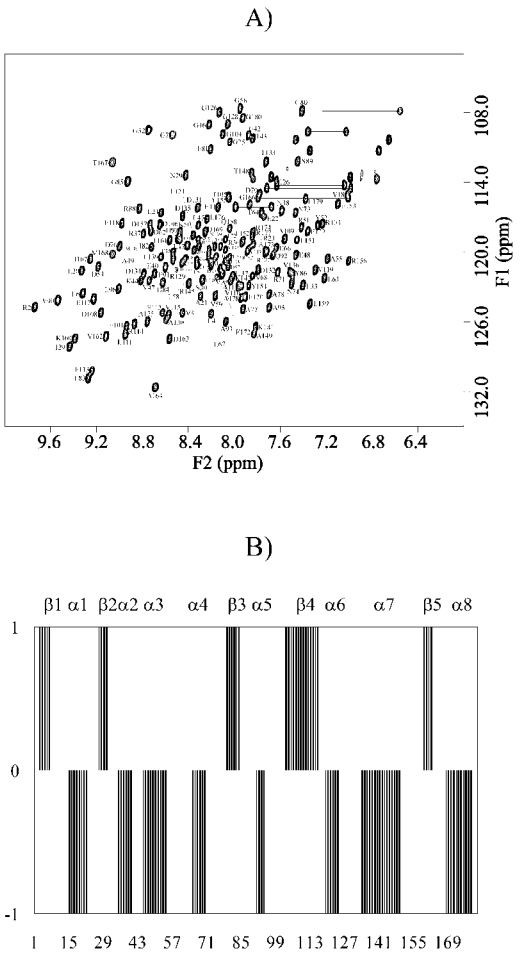

Figure 1. (A) 2D ^{1}H-^{15}N HSQC spectrum of *M. tuberculosis* adenylate kinase at 308 K. Assignments of resonances are indicated using one-letter codes for amino acids. Pairs of peaks connected by horizontal lines represent Asn and Gln side chain NH_2 groups. (B) The consensus chemical shift index (CSI) plot for AKmt obtained using $^{1}H^{\alpha}$,$^{13}C^{\alpha}$ and $^{13}C^{\beta}$ chemical shift values.

The NMR data were processed and analyzed using Felix98 software (MSI, San Diego, CA), running on a Silicon Graphics Indigo. The chemical shift indices (CSI) were obtained using the CSI software (Wishart and Sykes, 1994).

Extent of assignments and data deposition

The ^{15}N-HSQC spectrum (Figure 1A) of AKmt illustrates the good dispersion of the proton and nitrogen resonances in the amide groups.

^{1}H, ^{15}N, $^{13}C^{\alpha}$ backbone resonances and the side-chain $^{13}C^{\beta}$ resonances of 170 from the 174 non-proline residues as well as the ^{13}C resonances of 4 proline residues were assigned. Resonances corresponding to the segment G12–T15 were not observed, neither in the HSQC nor in the triple resonance spectra, and remain unassigned. The corresponding residues belong to the ATP binding P-loop (G10-T15), a flexible site which is considered to be in intermediate exchange (on the NMR chemical shift scale) between two or more conformations in the absence of any bound ligands (Burlacu-Miron et al., 1999).

The secondary structure prediction based on CSI (Figure 1B) and short-range NOEs analysis shows the existence of eight α-helices and five β-strands. The long-range NOEs indicate that these strands constitute a single parallel β-sheet. The topology of the β-sheet is similar to the other adenylate kinases, and only differs in the β5 strand, which is one residue shorter. The chemical shift values of the proton, nitrogen and carbon resonances have been deposited in the BioMagResBank (accession number: 4840).

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